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(54) **Doublestranded RNA correction of abnormalities in circulating immune complexes and monocyte function.**

(57) The use of a double-stranded RNA for the manufacture of a medicament for treating an inflammatory disorder characterised by elevated levels of circulating immune complexes in the patient's blood.

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DOUBLE-STRANDED RNA CORRECTION OF ABNORMALITIES IN CIRCULATING IMMUNE COMPLEXES AND MONOCYTE FUNCTION

A variety of major inflammatory disorders of man are characterized by circulating immune complexes (CIC), which are principally antibody-antigen complexes in serum. Persistent CICs are etiologically associated with a number of diseases, including systemic lupus erythmatosis (SLE), rheumatoid arthritis (RA), various malignancies, HIV, as well as infectious diseases due to other viruses, bacteria and parasites. The normal process of CIC clearance, i.e., removal by monocytes/macrophages, following their transport to the liver is also deranged in the above diseases plus other related disorders. Thus, associated disorders of peripheral blood monocyte functions and phagocytic capacity are commonly observed in these identical patient groups; collectively, these deficits may often lead to massive inflammatory destruction of various bodily tissues as well as decreased capacity to withstand various bacteria, viral and fungal pathogens to which these individuals are inevitably exposed.

I describe herein a novel method by which the majority of these functional defects can be controlled with a concurrent improvement in clinical status. Specifically, I describe a procedure, utilizing double-stranded RNAs, which simultaneously reduced CIC abnormal immune product depositions, and also improves monocyte function -- all untoward effects to the human subject. Also described are procedures for protecting thymus and bone marrow derived cells from virus infection and/or spread in which the subject is given an effective quantity of a matched or mismatched dsRNA capable of conferring viral resistance without adverse cellular toxicity.

BACKGROUND OF THE INVENTION

Usually beginning as a benign process of a normally functioning immune system, immune complex formation may initiate injury to various organs and tissues. The activation of complement, a normal blood product, by CICs can lead to a series of destructive events, including cell lysis and deposition of CICs on various vessel walls, cell membranes, etc. The antigens responsible for initiating such a pathogenic immune response are often unknown in specific clinical practice, but may be microorganisms, tumors or, indeed, the body's own tissues. Infectious diseases such as hepatitis B can also be accompanied by immune complex disease. CICs are so far reaching in relationship to diagnosis and measuring the efficiency of therapy in various clinical situations such that, in the last 10 years, more than 40 assays have been developed to detect and quantify such immune complexes in human pathologic fluids. Product brochures prepared by industry leaders, e.g., Maryland Medical Laboratory brochure, designated 4/87 or Roche Biomedical Laboratories brochure, prepared in 1987, indicate the range of the diseases associated with CICs and attest to the magnitude of the chemical problems secondary to enhanced CIC production in man.

CICs may actually contain virus particles as in the case of virus inclusion of the HIV itself in CICs of AIDS patients (Morrow et al, Clin. Immunology and Immuno Pathology, Vol. 40, p. 515, 1986). The CICs, especially if present in high concentrations, may actually cause an immunosuppressive effect and can even cause blockade of the body's vital reticuloendothelial system (see references cited in Morrow et al). Thus, circulating ICs may be one of the reasons for the abnormal functioning of monocytes-macrophages in many human and animal diseases. Accordingly, it is clear that, while formation of "small" amounts of IC may be part of a normal pathophysiological response to disease, inappropriate synthesis of IC will cause and/or accelerate various diseases.

Immune complex formation is a useful parameter particularly in assessment of rheumatic diseases, such as rheumatoid arthritis and systemic lupus. Specifically, elevated levels of immune complexes seem to track disease activity in many patients over time.

Among the methods used to evaluate CICs in man is the assay for the CR1 receptor, a glycoprotein that binds certain fragments (notably C3G and C4G) of the complement system. Erythrocytes or red blood cells (RBCs) carry on their surface the majority of CR1 receptors in the body's circulation. CR1 appears to endow RBCs with the capacity to clear CICs by transporting them to the liver where they are removed by local monocytes/macrophages (see Tausk et al, J. Clin. Investigation, Vol. 78, p. 977, 1986, and articles cited therein). Various diseases involving autoantibodies and CIC, such as arthritis, leprosy and AIDS, are associated with low levels of erythrocyte CR1.

DESCRIPTION OF THE INVENTION

In this invention, dsRNAs, preferably mismatched dsRNAs, are used to treat inflammatory disorders. An underlying cause of inflammatory disorders is the lack of availability of CR1 receptors and the raised levels of circulating C_3, as explained in detail below. These indicators are returned to normal by dsRNA treatment.

I evaluated a group of individuals with evidence of prior HIV infection to determine the various relationships between CIC level, CR1 level and disease severity (clinical status). Additionally, I employed other tests (referred to as the direct Coombs test) to determine if immune complexes were bound to the subjects' RBCs and the extent to which their reticuloendothelial systems, as probed by monocyte function and number, were operational upon completion of these base line measurements. I discovered that I could indeed reverse both the immunological derangements and deteriorated clinical status in a coordinate manner by administration of certain dsRNA molecules.

I first selected individuals with a prior history of HIV exposure, a prototypic immunological derangement, because (a) they have emerged as the prototype group manifesting high immune complex levels (see McDougal et al,J. Clin. Immun., Vol. 5, p. 130, 1985 and references cited therein), (b) the inciting (etiologic) microorganism is known, and (c) the disease is of unprecedented importance in the chronicles of public health. Finally, a variety of therapeutic modalities -- drugs and biologics -- (interferons, interleukins, thymus extracts, isoprinosine, thymus-derived polypeptide fractions, etc.) have been previously evaluated and none have shown any demonstrable ability to alter either the profound immune derangements, or the inexorable downhill clinical course. Finally, there are many compelling reasons that the identification of a novel therapeutic regimen, i.e., refraction of immune complex formation or increase in its clearance, would have profound ramifications in many other human disorders. Indeed, new evidence indicates that decremental loss of CR1 activity (due to progressive CIC formation) is associated with progression of retroviral/inflammatory diseases from an asymptomatic "carrier" state to a terminal moribund condition (see Inada et al, AIDS Research, Vol. 2, p. 235, 1986 and references cited therein).

By "matched dsRNA" are meant those in which hydrogen bonding (base stacking) between the counterpart strands is relatively intact, i.e., is interrupted on average less than one base pair in every 29 consecutive base residues. The term "mismatched dsRNA" should be understood accordingly.

The dsRNA may be a complex of a polynosinate and a polycytidylate containing a proportion of uracil bases or guanidine bases, e.g., from 1 in 5 to 1 in 30 such bases (poly I. poly (C_{4-29} x > U or G).

The dsRNA may be of the general formula $rI_n.r(C_{12},U)_n$. Other suitable examples of dsRNA are discussed below.

The mismatched dsRNAs preferred for use in the present invention are based on copolynucleotides selected from poly (C_n,U) and poly (C_n,G) in which n is an integer having a value of from 4 to 29 are are mismatched analogs of complexes of polyriboinosinic and polyribocytidilic acids, formed by modifying $rI_n.rC_n$ to incorporate unpaired bases (uracil or guanidine) along the polyribocytidylate (rC_n) strand. Alternatively, the dsRNA may be derived from poly (I). poly (C) dsRNA by modifying the ribosyl backbone of polyriboinosinic acid (rI_n), e.g., by including 2'-O-methyl ribosyl residues. The mismatched complexes may be complexed with an RNA-stabilizing polymer such as lysine or cellulose. These mismatched analogs of $rI_n.rC_n$, preferred ones of which are of the general formula $rI_n.r(C_{11-14},U)_n$ $rI_n1r(C_{29},G)_n$, are described by Carter and Ts'o in U.S. Patents 4,130,641 and 4,024,222 the disclosures of which are hereby incorporated by reference. The dsRNA's described therein generally are suitable for use according to the present invention.

Other examples of mismatched dsRNA for use in the invention include:

poly (I). poly (C_4,U)
poly (I). poly (C_7,U)
poly (I). poly (C_{13},U)
poly (I). poly (C_{22},U)
poly (I). poly (C_{20},G)
poly (I). poly (C_{29},G) and
poly (I). poly (C_p) 23 G>p

Also, as used in this application, the term lymphokines includes interferons, preferably interferon alpha, the interleukins, specifically interleukin-1 (IL-2) and recombinant interleukin-2 (rIL-2), and tumor necrosis factor (TNF). Also included are lymphokine activated killer cells (LAK) formed in animals in response to exposure to a lymphokine.

When interferon (alpha) is used as the lymphokine an amount of from 0.01 to 100,000 IRU per milliliter of the patient's body fluid is provided. When IL-2, preferably rIL-2, is the lymphokine, the amount

administered lies within a range of about 10^2 IL-2 units per kg of the patient's body weight up to a value approaching unacceptable levels of toxicity in that patient, which may be as high as 10^5 IL-2 units. However, most effective, toxic-reaction manageable values are in the range of from about 10^3 to about 10^4 IL-2 units per kg of body weight.

The usual amounts of dsRNA administered provide a level of from 0.1 to 1,000 micrograms dsRNa per milliliter of the patient's body fluid. Body fluid is intended to refer to that solution of serum, salts, vitamins, etc., which circulates within the organism and bathes the tissues When both agents are administered as previously described, the two active agents may be administered as a mixture, administered separately but simultaneously, or sequentially.

Administration of a dsRNA and a lymphokine "in combination" includes presentations in which both agents are administered together as a therapeutic mixture, and also procedures in which the two agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the drugs in which one of the drugs is given first and followed shortly by the second.

Restoration of Altered Immune Complex Formation by dsRNAs: Table 1 shows representative clinical results before, during and after administration of a dsRNA, $rI_nIr(C\cdots,U)_n$, 50-200 mg given twice a week to approximately 60 kilogram individuals. The Immune Complex test (Inada, 1986, reference cited above) looked for immune complexes capable of implementing the entire compliment activating process. Briefly, heat-inactivated patient serum and guinea pig complement were first combined and then reduced (using dTT, dithiothreitol) and, finally, added to normal RBCs as indicator cells with a known, predetermined content of free CR1 receptors. If immune complexes were present in the patient's serum, the indicator RBCs will aggregate; this is referred to as immune adherence hemagglutination of IAHA. I used the "free" CR1 receptor test to determine the level of "free" CR1 receptor activity on the patient's RBCs, e.g., compare the results in my Table 1 with those of Inada in various untreated individuals (as shown in his Figure 1, AIDS Research, Vol. 2, No. 3, p. 238, 1986)). I first mixed immune complexes, complement and the patient's RBC "ghosts", a technique described by Inada and well known to those in the art. If the patient's RBCs have available CR1 receptor, the CICs are bound such that when indicator RBCs (from normals) are added, no CICs are in free solution and the indicator RBCs do not aggregate. If degrees of aggregation do occur, it indicates that the patient's RBC ghosts were not able to bind CICs due to the absence of available CR1 receptors. The absence of CR1 receptors in this case appears to be due to the saturation of such receptors by the high (abnormal) level of circulating immune complexes. Low levels of "free" CR1 receptor is a bad prognostic sign and often correlates with a high level of CICs.

TABLE 1

Relationships Between CIC Levels, "Free" CR1 Receptor Content Direct Coombs' Test and the Effectiveness of dsRMA Administration on Correcting These Abnormal (Pathogenic) Immunologic Parameters				
Patient	Hematocrit	Immune Complex micrograms/ml	"free" CR1 Receptor	Direct Coombs' Test
1. Rejo, pretreat.	40	32	-	IgG, IgM, C_3
2 weeks	40	16	+	IgG, IgM, C_3
4 weeks	36	4	+ +	IgG, IgM, C_3
8 weeks	40	0	+ + +	IgG, IgM,
16 weeks	40	0	+ + +	trace IgG
2. Clin, pretreat.	45	40	-	IgM, C_3
2 weeks	46	20	+	O
4 weeks	45	0	+ +	O
8 weeks	45	0	+ +	O
16 weeks	45	0	+ + +	O
3. Tuja, pretreat.	36	36	+	IgG, IgM, C_3
4 weeks	34	28	+ +	IgG, C_3
8 weeks	35	16	+ + +	IgG, trace C_3
20 weeks	35	4	+ + +	O
40 weeks	35	0	+ + + +	O
4. Poca, pretreat.	34	32	-	IgG, IgM, C_3
8 weeks	36	4	+	IgG
20 weeks	36	4	+ +	trace IgM, trace C_3
40 weeks	36	0	+ + +	O
Normal Volunteers (10) tested neg. for immuno complex, + + + + for CR1 and neg. for Coombs				

One can readily determine that I was able to restore these parameters of inflammatory disease to essential normalcy by dsRNA administration. A comparison of Table 1 from the evidence given in the immediately preceding Table above, with Figure 1 and Table 1 of Inada et al clearly shows that my results with diseased patients, after dsRNA administration, very closely resembled the completely normal values of 37 healthy subjects reported by Inada et al with the same comprehensive laboratory parameters.

I have described above an overview of the Inada et al technique for "free" CR1 receptor which was used in the context of this invention. I also utilized the technique of Inada et al to measure actual types of immune molecules bound to the patient's RBCs (the direct Coombs' test) before, during and after dsRNA administration. Specifically, two classes of immunoglobulius -- designated IgG and IgM – when bound to RBCs are indicative of an active abnormal immune process. For example, Inada found no normal subjects with a positive direct Coombs' test, whereas 64-84% of patients with active retroviral infection were readily positive and degree of positivity correlated with clinical status. Also, I measured whether or not these RBCs were coated with a specific component of the complement system, designated C_3, which is also an additional reliable marker of an abnormal immune or inflammatory reaction. Specifically, the presence of C_3 on RBCs indicates an activation of the complement system or cascade in which C_1, C_4, C_2 and finally C_3 are sequentially bound to RBCs. Again, I demonstrated over time the completely unexpected finding that dsRNA could correct the abnormal concentration of inflammatory system components (e.g., IgG, IgM and C_3 molecules) from attaching to the patient's circulating RBCs.

Importantly, the kinetics of recovery of different limbs of the immune response in my work also showed that I was addressing with dsRNA an apparent root cause of the fundamental disease process. For example, immune complex level fell quickly (Table 1), correlating with the rapid clinical improvement whereas the direct Coombs' test improved more slowly. My observations support the thesis that (a) "wash out" of the abnormal Coombs' test is slow and often requires 2-3 months -- the approximate life span of the erythrocyte -- to clear, whereas (b) CIC levels fall more quickly because their appearance initially is usually only after saturation of the RBCs with abnormal immune complexes as measured by the direct Coombs' test. A comparison of my Table 1 (last column, direct Coombs' data) with Figures 2 and 3 of Inada presents

compelling evidence of the effect that I was indeed able to correct those profound immunologic derangements in my patient group to the essential normalcy as defined by immunological criteria developed completely independently by Inada et al with a completely healthy and normal population group, e.g., note that in Inada's group, no healthy subject had detectable immunoglobulin determinants or complement C_3 fragments on their RBCs.

Restoration of Altered Function of Blood Monocytes: Even though the functions of monocyte-macrophages play a central role in (a) initiation and modulation of immune response as well as in (b) defense mechanisms against microorganisma, these cells have been the subject of very few studies: see Roux-Lombard et al, European J. of Clin. Investigation, Vol. 16, p. 262, 1986 and references cited therein. For example, monocytes-macrophages can phagocytose (engulf) various microorganisms and produce reactive oxygen intermediates with high bactericidal potency. They also can secrete protaglandins which are known to alter T-cell functions. Thus, in view of their complementary and pivotal role in immune modulation vis à vis the other parameters I examined in Table 1, I studied both monocyte phagocytic and bactericidal capacity (a critical function) and number of peripheral cells before, during and after dsRNA administration in an identical group of individuals.

Mononuclear cells were obtained by me from heparinized blood by density gradient centrifugation in Ficoll-Hypaque. Their viability, as assessed by the trypan blue dye exclusion test, was greater than 95%. I then measured monocyte as well as T-lymphocyte subpopulations by use of standard monoclonal antibodies, including MO_2 (for monocytes), OKT_3, OKT_4, OKT_8, etc., followed by fluorescein-conjugated goat anti-mouse immunoglobulins obtained from Ortho Diagnostics, Inc., Raritan, New Jersey, U.S.A., to facilitate their enumeration.

TABLE 2

Restoration of Diminished Function/Number of Blood Monocytes in Individuals with Circulating Circulating Immune Complexes by dsRMA Administration

Patient	%Monocytes (MO2 Marker)	Number Monocytes (per cuomilliliter)	%Killed Bacteria (normal range = 10-62%)
1. Rajo, pretreat	1	55	0
2 weeks	6	330	4
4 weeks	12	660	10
8 weeks	18	990	18
16 weeks	17	935	
2. Clin, pretreat	2	126	3
2 weeks	8	504	10
4 weeks	13	819	35
8 weeks	15	945	40
16 weeks	19	1197	40
3. Tuja, pretreat	2	98	0
4 weeks	11	539	4
8 weeks	10	490	15
20 weeks	12	588	20
40 weeks	18	882	35
4. Poca, pretreat	1	40	2
8 weeks	9	315	16
20 weeks	12	420	24
40 weeks	20	700	40

I also evaluated bactericidal capacity by the standard technique using Staphylococcus aureus in the presence of either autologous or pooled type AB serum (Cruchard et al, Diagnostic Immunol., Vol 2, p. 203, 1984, the disclosure of which is hereby incorporated by reference). In Table 2, I express results as the percentage of bacteria that were killed after a 1 hour incubation.

Table 2 shows that there is indeed an unexpected and rapid recovery of both monocyte number and functions in the immunocompromised humans who received the scheduled administration of the mismatched dsRNA designated $rI_n \cdot r(C_{11-14}U)_n$, also called Ampligen® (a registered trademark of HEM

Research, Inc. of Rockville, Maryland, USA). Previous attempts by others to restore such function by use of other lymphokines have unfortunately resulted in failure. When Tables 1 and 2 are compared, it is apparent that I have accomplished a multifaceted effect in terms of restoring multiple limbs of the immunological capacity simultaneously, and I have accomplished this effect with favorable kinetics of response and apparent lack of host toxicity.

Table 3 shows that the protective effect of dsRNA extends well outside the various T cells components of the immune system. Here data are introduced to indicate effectiveness of dsRNA in protecting cells of bone marrow lineage such as, but not limited to, monocytes/macrophages. T cells are considered to have a thymus-derived lineage, hence I demonstrate here the additional capacity of certain dosages of dsRNAs to protect specifically human cells of both bone marrow and thymus derived origin; that is, the cells can be protected from animal viral infection without a detectable adverse effect on normal cellular functions including their replicative cycles. An HIV (virus) is used as a prototype because it is capable of mimicking other viruses either with acute cytolytic potential, subacute cytolytic potential, or even genomic integrative potential (latency and/or cancer-provoking potential).

Table 3 confirms the antiviral effect on various viral isolates (e.g., HTLV-III_B and HTLV III_{RF}) as well as various target cells (e.g., U937 or H9 cells).

Preventing and/or controlling infection in bone marrow derived cells without toxicity is highly relevant therapeutically since many animal viruses in fact use such cells as a primary or secondary viral source reservoir. For example, HIV may use macrophages, megakaryocytes and various cells which respond to colony stimulating factors as a significant aspect of its pathogenesis and ability to escape from the host's immunosurveillant capacities.

Claims

1. The use of a double-stranded RNA for the manufacture of a medicament for treating an inflammatory disorder characterised by elevated levels of circulating immune complexes in the patient's blood.

2. The use of a double-stranded RNA according to claim 1 in which the inflammatory disease is caused by a microorganism, such as a virus, a bacterium or a protozoan, an auto-or self-immune process, rheumatoid arthritis, hepatitis, or systemic lupus erythematosis.

3. The use of a double-stranded RNA according to claim 2 in which the inflammatory disease is classified as a Type A, a Type B or a non A/B hepatitis virus etiologic agent.

4. The use of a double-stranded RNA for the manufacture of a medicament for restoring a patient's immunological capacity.

5. The use of a double-stranded RNA according to claim 4 in which the mismatched double-stranded RNA is a poly I complexed with a poly C, preferably one in which the poly I complexed with the poly C is additionally complexed with an RNA-stabilising polymer such as lysine or cellulose.

6. The use of a double-stranded RNA according to any one of claims 1 to 4 in which the double-stranded RNA is polyadenylic acid complexed with polyuridylic acid , preferably mismatched.

7. The use of a double-stranded RNA according to any one of claims 1 to 4 in which the double-stranded RNA is mismatched and is a complex of a polyinosinate and a polycytidylate containing from 1 in 5 to 1 in 30 uracil or guanidine bases, and preferably the mismatched dsRNA is $rI_n.r(C_{11-14},U)_n$.

8. The use of a double-stranded RNA according to any one of the preceding claims wherein the medicament comprises a combination of a double-stranded RNA and a lymphokine such as an interferon or an interleukin.

9. The use of a double-stranded RNA for the manufacture of a medicament for protecting thymus and bone marrow-derived cells from virus infection or spreading virus, wherein the double-stranded RNA is matched or mismatched and is capable of conferring viral resistance without adverse cellular toxicity.

10. The use of a double-stranded RNA according to claim 9 in which the RNA is mismatched and is poly I complexed with poly C.

11. The use of a double-stranded RNA according to claim 10 in which the poly I complexed with poly C is additionally complexed with an RNA-stabilizing polymer.

12. The use of a double-stranded RNA according to claim 11 in which the stabilizing polymer is lysine or cellulose.

13. The use of a double-stranded RNA according to claim 9 in which the dsRNA is polyadenylic acid complexed with polyuridylic acid.

14. The use of a double-stranded RNA according to claim 9 in which the double-stranded RNA is mismatched.

15. The use of a double-stranded RNA according to claim 14 in which the mismatched double-stranded RNA is a complex of a polyinosinate and polycytidylate containing from 1 in 5 to 1 in 30 uracil or guanidine bases.

16. The use of a double-stranded RNA according to claim 15 in which the mismatched double-stranded RNA is $rI_n.r(C_{11-14}.U)_n$.

17 The use of a double-stranded RNA according to claim 9 in which the double-stranded RNA contains regions of bond breakage and the double-stranded RNA exhibits the favourable therapeutic ratio property of $rI_n.r(C_{11-14}.U)_n$.

18. The use of a double-stranded RNA according to claim 14 in which the medicament contains mismatched double-stranded RNA in an amount which will result in a level of from 1 to 1000 micrograms per milliliter of the patient's body fluid.

19. A method of diagnosing an inflammatory disorder induced by circulating immune complexes in the patient's blood comprising assessing the circulating immune complexes in a sample of a patient's blood serum by (a) determining the level of available CR1 receptors on the red blood cells of a patient sample, (b) conducting a direct Coomb's test to determine the types of immune molecules bound to the patient's red blood cells, or (c) assessing the level of the patient's red blood cells coated with C_3 as a measure of activation of the patient's complement system.

20. A method of diagnosing and restoring a patient's immunological capacity comprising assessing the patient's immunological capacity by (a) collecting mononuclear cells from the patient, measuring the monocyte and T-lymphocyte subpopulation, and comparing the measured results to those of a known, standardized, asymptomatic population; (b) measuring the bacterial killing capacity of a patient's blood sample and comparing the measured result to those of a known, standardized, asymptomatic normal population; or (c) both (a) and (b).

AMPLIGEN EFFECT *IN VITRO* ON AN HIV PERMISSIVE HUMAN MONOCYTE/MACROPHAGE CELL LINE (U937)

Culture Conditions[a]	DAY 2	4	6	8	10	12	14	
U937/HTLV-III_B No Ampligen	—	—	9.6×10^4	2.0×10^6	1.6×10^7	5.1×10^6	—	RT Activity (cpm/ml)[b]
	—	3 - 5	15 - 18	40	100	100	—	% IIF[c]
U937/HTLV-III_B Ampligen (50 µg/ml)	—	—	8.0×10^3	3.0×10^4	1.6×10^5	1.0×10^6	—	RT Activity (cpm/ml)
	—	<1	1	2 - 3	5	30	—	% IIF
H9/HTLV-III_{RF} No Ampligen	—	—	3.8×10^4	1.6×10^6	1.8×10^7	9.6×10^6	—	RT Activity (cpm/ml)
	—	1 - 2	5	20 - 25	100	100	—	% IIF
H9/HTLV-III_{RF} Ampligen (50 µg/ml)	—	—	0	0	2.4×10^4	2.4×10^5	—	RT Activity (cpm/ml)
	—	<1	<1	<1	2 - 4	5 - 7	—	% IIF
H9/HTLV-III_B No Ampligen	—	—	0	3.0×10^4	8.6×10^5	7.2×10^6	—	RT Activity (cpm/ml)
	—	1	1 - 2	5 - 7	20 - 30	100	—	% IIF
H9/HTLV-III_B Ampligen (50 µg/ml)	—	—	0	0	0	5.6×10^4	—	RT Activity (cpm/ml)
	—	≪1	≪1	<1	1	5 - 7	—	% IIF

a *Producer cell/HIV-1 isolate using U937 cells as targets with or without Ampligen as indicated.*

b *RT = Reverse transcriptase*

c *IIF = Indirect immunofluorescence expressed as % positive cells using a human anti-p24 serum.*

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